A review on research progress of transketolase

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Abstract: Transketolase (TK), a thiamine diphosphate (ThDP)-dependent enzyme, catalyzes several key reactions of non-oxidative branch of pentose phosphate pathway. TK is a homodimer with two active sites that locate at the interface between the contacting monomers. Both ThDP and bivalent cations are strictly needed for TK activation, just like that for all ThDP-dependent enzymes. TK exists in all organisms that have been investigated. Up to now, one TK gene (TKT) and two transketolase-like genes (TKTL1 and TKTL2) have been identified in human genome. TKTL1 is reported to play a pivotal role in carcinogenesis and may have important implications in the nutrition and future treatment of patients with cancer. Researchers have found TK variants and reduced activities of TK enzyme in patients with neurodegenerative diseases, diabetes, and cancer. Recent studies indicated TK as a novel role in the prevention and therapy of these diseases.

Keywords: Transketolase; Pentose phosphate pathway; neurodegenerative disease, TKTL-1 gene

There are mainly two biochemical pathways known as Embden-Meyerhof pathway and Pentose Phosphate Pathway (PPP) in glucose metabolism. Embden-Meyerhof pathway was identified in the 1930s based on the fact that muscle extracts could catalyze the transformation from glucose to lactate. PPP is a multifunctional pathway, also known as the phosphogluconate pathway or the hexose monophosphate shunt. The first function of PPP in mammalian cells is to produce reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) which functions in detoxification processes and lipid biosynthesis. The second function is to convert hexose into pentose, particularly into ribose-5-phosphate which is required for nucleic acids synthesis. Transketolase (TK) catalyzes several reactions in the non-oxidative branch of the PPP and serves as a bridge between

the oxidative part of PPP and oxidative decarboxyl metabolism of glucose, allowing the cell to adapt to a variety of metabolic needs under different environmental conditions^[1]. Studies have demonstrated that TK activity was related to neurodegenerative diseases^[2,3], diabetes^[4], and cancer^[5].

1 The structure of TK

As a thiamine diphosphate (ThDP)-dependent enzyme, TK is a homodimer with two active sites located at the interface between the contacting surfaces of the monomers^[6]. TKs purified from yeasts and mammalian tissues have been reported to contain one molecule of ThDP per subunit^[7], whose crystal structure has been confirmed^[8]. ThDP exerts a considerable stabilizing effect on apo-TK and is indicative of a more compact structure of holo-TK compared to that of apoTK^[9]. Like other ThDP-dependent enzymes, TK activation requires ThDP and bivalent cations simultaneously. The bivalent cation is indispensable to TK activity, which serves as a bridge between apoTK and ThDP^[6].

2 Originations and properties of TK

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Fig. 1 Three-dimension structure of TK (adapted from Sundström M, *et al.* 1992)^[10]

2.1 Distribution of TK TK exists in all organisms that have been investigated. The enzyme can be purified from various tissues, including baker's yeast, *Candida utilis*, spinach, pig liver, rat liver, rabbit liver, mouse brain, human leukocytes and human erythrocytes. Its distribution and metabolic activity in higher organisms are tissue-dependent. Both distribution and activity are low in skeletal muscle and liver, while much higher in other tissues, including adipose tissue, mammary glands, the adrenal cortex, and so on where large amounts of NADPH are required for the synthesis of fatty

acids and steroids from acetyl CoA^[11].

2.2 Substrate specificity A broad range of substrates for the TKs from yeasts, plants and bacteria have been reported. However, mammalian TKs are more specific, recognizing only D-xylulose 5-phosphate, D-fructose 6-phosphate and D-sedoheptulose 7-phosphate as donors, while D-ribose 5-phosphate, D-erythrose 4-phosphate, D-glyceraldehyde 3-phosphate and glycolaldehyde are recognized as acceptor substrates^[12].

2.3 TK stability and the optimum pH for its activity Although repeated freezing and thawing causes damage to erythrocyte TK activity, native human TK is remarkably stable. TK remains active over two weeks undergoing purification at 24 °C. Human TK purified from brain tissues retains 80%-90% of its initial activity after storage at -20 °C for one month^[13]. The optimum pH range for human TK is between 7.5 and 10, which is similar for all kinds of TK^[14].

3 Metabolic roles of TK

TK, together with transaldolase (TA), catalyses reactions in the non-oxidative branch of PPP. TK catalyses the conversion of D-pentose (xylulose and ribose) 5-phosphate into D-glyceraldehyde 3-phosphate and D-sedoheptulose 7-phosphate. TK also catalyses the conversion of D-xylulose 5-phosphate and D-erythrose 4-phosphate into D-glyceraldehyde 3-phosphate and D-fructose 6-phosphate^[11]. The reactions shown in Fig. 2 are based on the results of catalysis by liver enzyme incubated with ¹⁴C-substrates of various pentose phosphate. The conclusion concerning the nature

Fig. 2 The non-oxidative branch of the pentose phosphate pathway catalysed by TK and TA (adapted from Schenk G *et al.* 1998)^[11].

and order of these reactions is still controversial because ^{14}C isotope from donor substrates could be converted to carbon atom 1 and 3 of receptor substrates, such as fructose-6-phosphate, which is different from theoretical prediction by the reaction sequences shown in Fig. 2^[15]. To explain the discrepancy between the proposed reaction sequences and the observed results, a mathematical model has been developed^[16], but further experimental analysis is still needed.

4 TK gene family

The first report of TK isomer was published 30 years ago^[17]. The chromatographic analysis of human erythrocyte TK found that the enzyme activity was divided into three peaks^[18]. Hitherto, one transketolase (TKT) and two transketolase-like genes (TKTL1 and TKTL2) have been identified in human genome^[19]. TKTL1 gene, which locates in Xq28, a region that contains the genes activated in malignancies and the cell-cycle, plays an important role in carcinogenesis^[20].

5 Association of TK with diseases

5.1 TK and Wernicke's encephalopathy (WE) Thiamine deficiency (TD) in human leads to Wernicke's encephalopathy (WE), a neuropsychiatric disorder commonly encountered in chronic alcoholism and in patients with impaired nutrition associated with gastrointestinal disease or AIDS^[21]. Histopathologic evaluation of brain tissues from WE patients revealed neuronal loss, gliosis and vascular damage in mid-line regions of the brain, including the mammillary bodies, inferior olive, thalamus and other periventricular regions^[22]. Like other ThDP-dependent enzymes, TK activity is decreased in conditions of thiamin deficiency. Among all the ThDP-dependent enzymes in the brain, TK activity decreases first and returns the latest following thiamin depletion in thiamin-deficient animals^[2]. Our unpublished data demonstrated the involvement of both decreased TK activity and dysfunction of pentose phosphate shunt in the pathogenesis of brain regionally selective damage of WK. However, Victor *et al.* reported that decreased TK activity did not directly lead to the focal neuropathology since the activity change correlated poorly with alterations in the disease manifestation^[23].

Numerous mechanisms have been proposed to explain the pathogenesis of WE, in the hope of finding more effective therapy for WE. These include cerebral energy dysfunction^[24], breakdown of the blood-brain barrier^[25], altered glutamate neurotransmission caused by impaired transport function^[26], N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity^[27], the accumulation of amyloid precursor-like protein^[28], increased free radical production^[29], increased expression of superoxide dismutase consequent to increased microglial response^[30], induction of nitric oxide^[31], oxidative stress^[32] and so forth. However, it remains to be studied which one is of primary importance. It is also possible that multiple genetic defects or combination of several processes are involved in the pathogenesis of WE.

5.2 TK and Alzheimer's disease Postmortem examination in brains and other tissues from the patients with Alzheimer's disease (AD) showed decreased TK activity^[3]. Variants of TK protein with different isoelectric points have been detected in AD skin fibroblast cells. This change could be attributed to proteolytic cleavage of TK producing a small sub-unit that retained some activity^[33]. TK is abnormally and characteristically degraded during extraction of AD fibroblasts. In contrast, TK from patients with other neurological diseases and control subjects remains unaltered in activity^[33]. These changes in TK activity might represent a marker for Alzheimer's disease, even some of the asymptomatic relatives of AD patients could exhibit TK alterations. Further investigations on this early biochemical marker lead to the conclusion that lysosomal cathepsins, particularly cysteine proteinases, are involved and could be the more relevant research targets for both pathogenetic and diagnostic purposes^[34]. Interestingly, TK alterations in AD fibroblasts could be abolished by the treatment with cysteine proteinase inhibitors or the acidotropic agent NH_4Cl , which impairs the function of the endolysosomal compartment^[35].

5.3 TK and diabetes Evidences have strongly indicated that TK is also important in diabetes. A high blood glucose level induces concomitant high glucose concentrations in retinal, endothelial and neuronal cells. Under high blood glucose level, glucose and other reduced sugar react nonenzymatically with protein amino groups to initiate a post-translational

modification process known as nonenzymatic glycation^[36]. Glycation gives rise to advanced glycation end products (AGEs), thereby causes vascular complications, neuropathy and retinopathy. These pathological alterations can be ameliorated by taking vitamin B1. The lipid-soluble thiamine derivative benfotiamine activates TK and prevents diabetic retinopathy through blocking three major pathways of hyperglycemic damage^[4]. Therefore, TK may be a new target for prevention and treatment of chronic diabetes complications.

5.4 TK and cancers The non-oxidative branch of PPP catalyzed by TK plays a crucial role in utilizing glucose carbons for ribose-5-phosphate synthesis in tumor cells. More than 85% of ribose for nucleoside synthesis in certain tumor cells derives directly or indirectly from the nonoxidative part of PPP^[5]. It has recently been reported that TK and its cofactor thiamine have a very high growth control coefficient in the Ehrlich tumor model^[37]. Researchers found a strong upregulation of the mutated transketolase transcript (TKTL1) in human hepatoma cell line HepG2, while TKT and TKTL2 transcripts were not upregulated^[38]. Langbein *et al.* reported that TKTL1 expression was correlated to poor prognosis of invasive colonic and urothelial tumors^[39]. Tumor cells with an upregulation of TK exhibit enhanced non-oxidative glucose usage and, therefore, are well adapted to the anoxic environments^[40]. The importance of TK for tumor cell metabolism is underlined by the fact that the application of specific TK inhibitor (Oxythiamin) induces a dramatic suppression in tumor proliferation^[41]. In addition, metabolic control analysis indicates that stimulative factors of TK synthesis, such as dietary thiamine, can support tumor cell survival and proliferation. This may have crucial implications in the nutrition and future treatment of patients with cancer^[42].

6 Conclusion

This review highlights the structure and some properties of TK and its relationship with several diseases. TK plays a critical role in the metabolism of organisms and has broad research prospect. Although some published data are still controversial, they indicate a new explorative direction for the associated diseases. Meanwhile, further research is still required to establish novel therapeutical strategy for AD,

diabetes, and cancer.

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转酮醇酶的研究进展

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摘要: 转酮醇酶(Transketolase, TK)是一种焦磷酸硫胺素(thiamine diphosphate, ThDP)依赖性酶, 负责催化磷酸戊糖通路中碳水化合物转化的一个关键反应。此酶属于同源二聚体, 在单体间的接触界面上存在两个活性部位。与所有的焦磷酸硫胺素依赖性酶一样, TK 活性不仅依赖于焦磷酸硫胺素的存在, 还需要二价阳离子。TK 存在于所有研究过的生物体中。迄今为止, 在人类基因组中, 已经确定了一个 TK 基因和两个 TK 相似基因, 即转酮醇酶基因(TKT)、转酮醇酶样基因-1 (transketolase like-1, TKTL-1)和转酮醇酶样基因-2 (transketolase like-2, TKTL-2)。据报道, TKTL-1 在肿瘤发生中起着重要的作用, 同时对肿瘤患者的营养搭配及未来的治疗等方面有着重要的提示。在神经变性疾病、糖尿病和癌症中均发现转酮醇酶样基因变异体的存在, 转酮醇酶的活性也有所降低。这些资料为更好地研究这些疾病的发病机理提供了新的线索, 并有助于建立新的预防和治疗手段。

关键词: 转酮醇酶(TK); 磷酸戊糖途径; 神经变性疾病; TKTL-1 基因